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Isolation and expression of ACC oxidase in *Hevea brasiliensis*

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Kuswanhadi

Application of ethephon to a rubber tree tapping panel.

Ethephon, an ethylene generator, is applied to the bark of rubber trees to increase rubber production by stimulating latex regeneration and flow. However, a good command of both the stimulation frequencies and the ethephon concentrations to be used during tree tapping is required to avoid oxidative burst that can result in tapping panel dryness, leading to a loss of production. Studies on ethylene biosynthesis and its regulation were needed to gain a better understanding of the mechanisms involved in latex production. Hence, ACO genes were isolated from the rubber tree and characterized by semi-quantitative RT-PCR.

Kuswanhadi

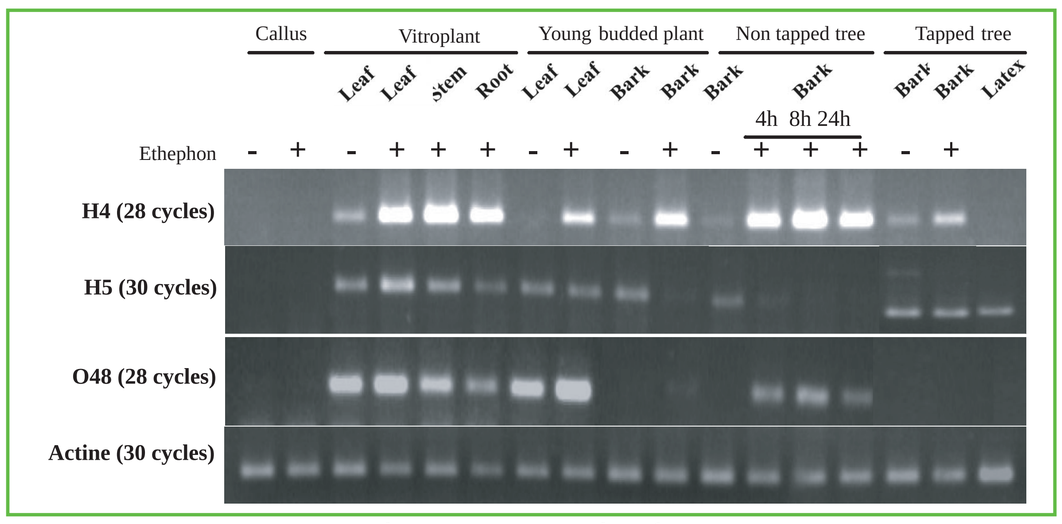
Rubber tree tapping.

Isolation of three genes encoding ACC oxidase

Total RNA was extracted from various tissues of clone PB 260 in a guanidium thiocyanate solution by ultracentrifugation through a cesium chloride cushion. Using degenerate primers, partial fragments were amplified by PCR and then cloned. Full length cDNAs were isolated either by cDNA library screening (clone H5) or by RACE technology (clones H4 and O48). *HbACO-H4*, *HbACO-H5*, and *HbACO-O48* encoded polypeptides of 318, 315, and 318 amino acids respectively, having 79 to 92% protein identity and 75 to 86% nucleotide homology between them. Two genomic sequences were isolated. First *HbACO-H4*, which was 1504 bp long and consisted of 2 introns and 3 exons. Then *Hb-ACO-H5*, which was 1456 bp long and consisted of 3 introns and 4 exons. These results suggested that we had isolated three members of this multigenic family.

Expression of *HbACO* genes during plant development

This characterization at several stages of development revealed differential regulation of their expression (Figure 1). *HbACO-H4* was strongly expressed in all tissues but latex when ethephon was applied. Conversely, O48 expression was more restricted to juvenile material and its response to ethephon application was lower. Although *H5* was slightly expressed, it was mostly found in all tissues and did not respond to ethephon stimulation.



Treatments: (-) no stimulation, (+) 24 h after ethephon stimulation.

Figure 1. Expression of *HbACO* genes during plant development.

Expression of *HbACO* genes in response to ethephon or ethylene

Young grafted plants were treated with 2.5% ethephon or 1 ppm ethylene and the kinetics of gene expression were analysed from 1 to 168 hours. The expression of *HbACO-H4* and *HbACO-O48* was induced by both treatments, whereas *HbACO-H5* tended to be down-regulated in bark (Figure 2) and leaf tissues (Figure 3). According to the number of PCR cycles, member O48 was the most expressed, its expression being more transient in bark than in leaves, where it was still expressed after 168 hours.

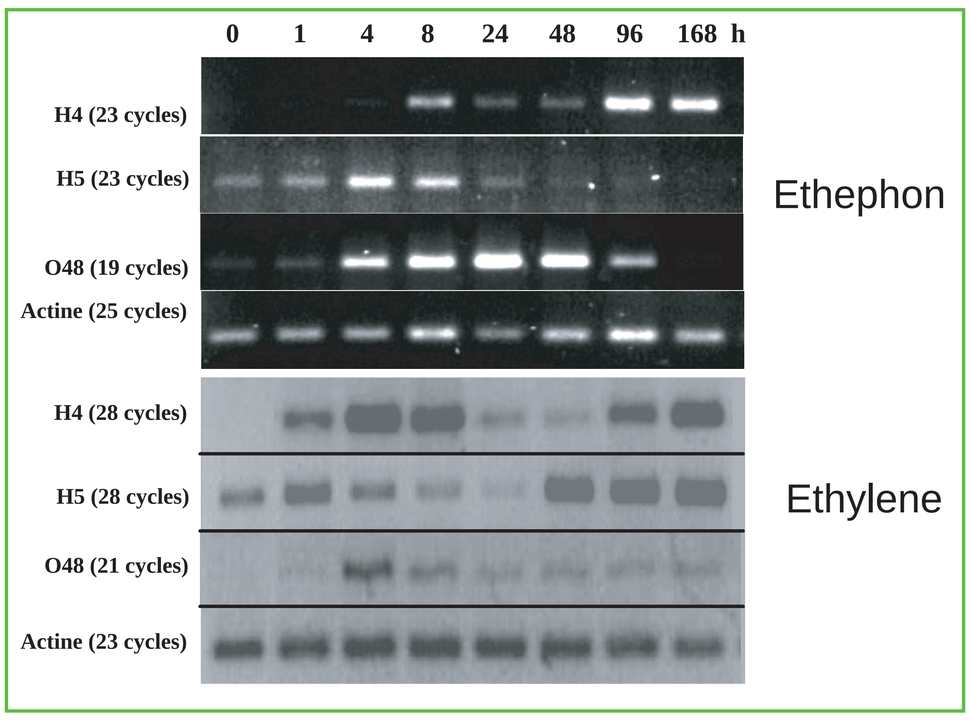


Figure 2. Expression of *HbACO* genes in bark tissues in response to ethephon or ethylene.

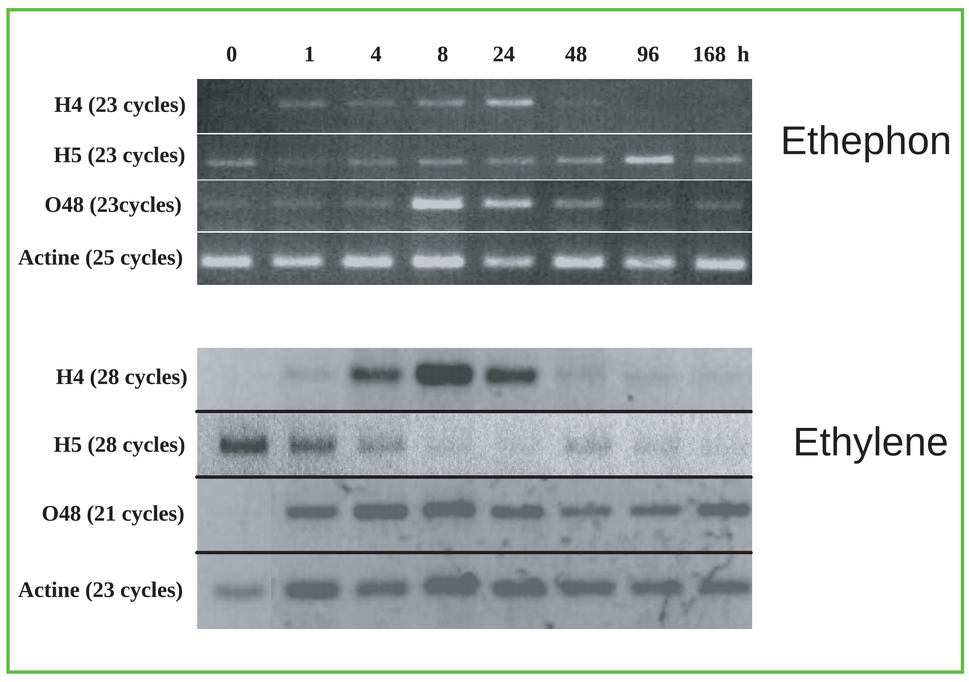


Figure 3. Expression of *HbACO* genes in leaf tissues in response to ethephon or ethylene.

Conclusion

The multigenic family encoding ACOs consisted of at least three members in the genome of *Hevea brasiliensis* clone PB 260. Their characterization at several stages of development revealed differential regulation of their expression: *HbACO-H4* was induced by ethephon in roots, leaves and bark tissues; *HbACO-H5* displayed low but constitutive expression; and *HbACO-O48* was more expressed in juvenile tissue. None of them was expressed in callus. A kinetics analysis on young grafted plants confirmed these results but revealed very transient expression of O48 in bark, which could not be detected after 24 hours. Gene expression patterns were similar in response to ethephon and ethylene, although ethylene action was faster. At that stage, *HbACO-O48* was the most strongly expressed gene in both bark and leaves. These observations tallied well with the fact that Ethrel application triggers endogenous ethylene production via autocatalytic reactions. The very weak expression found in latex tended to show that the seat of ethylene biosynthesis would appear to be in bark tissues in the vicinity of laticifer cells.

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